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Imaging of Cardiac Allograft Rejection in Dogs Using Indium-111 Monoclonal Antimyosin Fab

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The acute rejection of cardiac allografts is currently diagnosed by the presence of myocyte necrosis on endomyocardial biopsy. We evaluated the efficacy of noninvasive scintigraphic imaging with indium-111–labeled anticardiac myosin Fab fragments (indium-111 antimyosin) to detect and quantify cardiac allograft rejection. Six dogs that had intrathoracic heterotopic cardiac allograft transplantation were injected with indium-111 antimyosin and planar and single photon emission computed tomographic (SPECT) images were obtained in various stages of acute and subacute rejection. Four dogs had an allograft older than 8 months and had been on long-term immunosuppressive therapy; two dogs had an allograft less than 2 weeks old and were not on immunosuppressive therapy. Count ratios comparing heterotopic with native hearts were calculated from both SPECT images and in vitro scans of excised and sectioned hearts and were compared with the degree of rejection scored by an independent histopathologic review.

Indium-111 antimyosin uptake was not visible in planar or SPECT images of native hearts. Faint diffuse uptake was apparent in cardiac allografts during long-term immunosuppression and intense radioactivity was present in hearts with electrocardiographic evidence of rejection. The heterotopic to native heart count ratios in SPECT images correlated significantly with the count ratios in the excised hearts ($r = 0.93$) and with the histopathologic rejection score ($r = 0.97$). The distribution of indium-111 antimyosin activity in right and left ventricles corresponded to areas of histopathologic abnormalities. Immunoperoxidase studies showed deposition of indium-111 antimyosin only in areas of myocyte necrosis. The results demonstrate that indium-111 antimyosin imaging can noninvasively detect the presence, location and severity of canine cardiac allograft rejection.

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Cardiac transplantation is becoming an increasingly accepted form of therapy for end stage cardiac disease. Improved success with this treatment has been attributed to the use of cyclosporine immunosuppression (1). The International Heart Transplantation Registry recently reported an 80% 1 year survival rate after cardiac transplantation using cyclosporine immunosuppression compared with 60% using conventional azathioprine and prednisone therapy (2). The use of cyclosporine, however, makes the diagnosis of cardiac allograft rejection more difficult. The current definitive method for the diagnosis of cardiac allograft rejection in patients receiving cyclosporine immunosuppression is the endomyocardial biopsy and the pathologic hallmark of significant rejection is myocyte necrosis (1,3). Cardiac biopsy, however, is invasive and inconvenient. Currently available noninvasive methods are either nonspecific indicators of myocardial dysfunction or have not proved clinically useful in specifically diagnosing allograft rejection (3–5).

Khaw, Frame and colleagues (6–9) developed the method for detecting and localizing a myocardial infarct employing intravenously administered radiolabeled fragments of antibodies specific for cardiac myosin. In an extensive series of experiments, they found that labeled polyclonal anticardiac myosin antibodies, $F(ab')_2$ and Fab fragments of these antibodies localize in myocardial cells irreversibly damaged by an ischemic insult. More recently (10) they used hybridoma technology to produce monoclonal antibodies to

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human cardiac myosin. Conjugation of Fab fragments of these monoclonal antibodies to the bifunctional chelating agent, diethyelenetriamine pentaacetic acid, facilitated radiolabeling with either technetium-99m or indium-111. Thus, a monoclonal antibody Fab fragment specific for human cardiac myosin labeled with a radionuclide was produced for imaging of myocardial infarction in vivo by gamma scintigraphy (10).

The purpose of our study in experimental animals was to test whether the scattered myocyte necrosis that occurs on a microscopic level during cardiac allograft rejection can be detected and localized scintigraphically using indium-111–labeled monoclonal antimyosin Fab fragments (indium-111 antimyosin). A secondary objective was to determine whether the degree of Fab uptake visualized on the scintigrams was related to the severity of allograft rejection determined by histopathologic examination. Successful application of such scintigraphic approaches may ultimately lead to a noninvasive test for cardiac transplant rejection that could be an effective alternative to endomyocardial biopsy.

Methods

Animal model. Six adult mongrel dogs that had undergone intrathoracic heterotopic cardiac allograft transplantation were studied. This experimental model was chosen so that the native heart of each dog could serve as a control for the transplanted heart. Cardiac transplantation was performed according to the method described by Jamieson et al. (11). Outbred mongrel donor dogs weighing 9 to 14 kg were matched with recipient dogs weighing 14 to 23 kg. Donor and recipient pairs were anesthetized with sodium pentobarbital and were ventilated with a Harvard respirator. The donor heart was arrested with cold crystalloid cardioplegia solution and harvested through a median sternotomy. A left thoracotomy was performed on the recipient and after creation of an atrial septal defect and mitral incompetence in the donor heart, the donor aorta and pulmonary artery were anastomosed end to side to the recipient left innominate artery and superior vena cava, respectively.

Antibody preparation. Monoclonal anti-human cardiac myosin antibodies that also cross-react with canine cardiac myosin were prepared from murine sera and were purified by Centocor, Inc. These antibodies were originally developed by Khaw et al. (10). Using the technique of somatic cell fusion, spleen cells from Balb/c mice immunized with human cardiac myosin were fused with a plasmacytoma cell line to make the hybrid cell line R11D10. Fab fragments of the IgG antibody produced by these cells were prepared by mercuripapain digestion and then covalently attached to DTPA. The resultant Fab-DTPA molecules were suspended in 0.1 *M* sodium citrate buffer in preparation for imaging.

Imaging protocol. The preparation of the indium-111-labeled Fab for imaging involved incubation of 2 to 3 mCi of indium-111 chloride with the citrate-buffered antimyosin Fab-DTPA at room temperature. The percent of isotope binding to protein was determined using paper chromatography. All injectates used in this study had more than 95% of the indium-111 bound to the protein. The dissociation of the indium from the antimyosin occurs at less than 1% per day until the antibody is cleared.

Imaging was performed 24 hours after the intravenous injection of 2 to 3 mCi of indium-111 antimyosin. The 24 hour time interval was chosen based on blood clearance curves that showed a slow disappearance of indium-111 antimyosin with the curve reaching a low steady state by 24 to 48 hours. A dual-headed tomographic imaging system (Picker International) with matched sodium iodide detectors and medium energy parallel hole collimators was used to obtain single photon emission tomographic images (SPECT). Pulse-height analyzer windows were set to record both the 169 and 240 keV photopeaks of indium-111 with a 15% window width. The dogs were anesthetized with 30 mg/kg body weight intravenous sodium pentobarbital. They were intubated but allowed to breathe spontaneously and were placed in the right lateral decubitus position on the pallet of the SPECT camera. Data were acquired by each camera head for 1 minute at each of 30 projections as the gantry rotated in a 180° circular arc, yielding a 360° acquisition with 60 total projection images. Typical images contained 150,000 to 250,000 counts. Total scanning time was 30 minutes.

Through a direct digital interface, a dedicated minicomputer (A^3, Medical Data Systems) stored the data in a 64 × 64 word matrix using 1.33× software magnification for later computer reconstruction. Subsequent 5 minute static acquisitions in the anterior and left lateral positions also were obtained. These images contained approximately 500,000 to 750,000 counts.

Thallium-201 imaging was performed immediately after indium-111 imaging to help delineate both cardiac borders in each dog. Thallium-201 chloride (2 mCi) was injected and planar and SPECT images were acquired after a 10 minute equilibration period using the same protocol described for indium-111 imaging. The 70 keV photopeak of thallium-201 and a 15% window were used for acquisition of these scans. Total counts in thallium-201 images were approximately 75% of the total counts in corresponding indium-111 views.

Immunosuppression protocols. To test the effectiveness of indium-111 antimyosin in detecting and quantitating rejection over a range of histopathologic rejection grades, the dogs were studied according to several protocols (Table 1).

As a model of severe early rejection, two animals (Dogs

Table 1. Heterotopic Cardiac Allograft Imaging Sequence

	Time From Transplant to Scan	Immunosuppression	Baseline Scan	Rejection Scan	Immunosuppression for Second Scan	Time Between Scans
Acute						
Dog 1	10 Days	No	—	Yes		—
Dog 2	4 Days	No	—	Yes		—
Chronic						
Dog 3	8 Mo	Yes	Yes	No	—	—
Dog 4	9 Mo	Yes	Yes	Yes	No	16 Days
Dog 5	8 Mo	Yes	Yes	Yes	No	27 Days
Dog 6	10 Mo	Yes	Yes	Yes	Yes	14 Days

1 and 2) underwent cardiac transplantation and the hearts were allowed to reject without immunosuppression. Thallium-201 and indium-111 antimyosin scans were obtained on one donor and recipient dog pair before surgery to look for possible indium-111 antimyosin uptake in normal hearts and to provide a baseline for comparison with later scans. After transplantation, daily electrocardiograms were performed until allograft rejection was suspected, as evidenced by a 25% decrement in the R wave voltage ratio of the transplanted and the native heart, QRS wave widening or significant arrhythmia. When electrocardiographic evidence of rejection occurred, indium-111 antimyosin and thallium-201 scans were performed as described under Imaging Protocol and the dogs were killed for pathologic examination.

The four remaining dogs (Dogs 3 to 6) represented long-term transplantation survivors on chronic immunosuppressive therapy. This model was selected to evaluate whether operative trauma affected the antimyosin antibody imaging. Dogs in this group had undergone heterotopic transplantation more than 8 months before study and had been maintained on prednisone (5 mg/day) and azathioprine (2 mg/kg per day) immunosuppression since surgery. Azathioprine immunosuppression was chosen for our long-term dog models because it is possible to clinically detect early rejection with this regimen using electrocardiographic changes as criteria. In this way we could more accurately determine when to perform the second scans.

Dog 3 was given an injection, scanned and killed while receiving this long-term immunosuppressive therapy and without electrocardiographic evidence of rejection to serve as a control for the other long-term dogs. *Dogs 4 and 5* were each studied twice to characterize the changes in scans that occur with the development of rejection. In these dogs, baseline scans were performed while they were receiving immunosuppressive therapy; after that the medications were discontinued and the dogs were allowed to develop allograft rejection. When electrocardiographic changes suggestive of rejection were present, the dogs were given another injection, indium-111 antimyosin, rescanned and killed. *Dog 6*, also on long-term immunosuppression, spontaneously began to reject according to electrocardiographic criteria on the day of the initial scan. Because this dog was clinically well, this episode of rejection would not have been detected had the dog not been entered into the protocol. This animal, therefore, represented a model of undetected rejection in the presence of immunosuppressive therapy. After the baseline scan was performed, Dog 6 was maintained on treatment at the same doses for 2 weeks and was then restudied and killed.

Data analysis. All single photon emission computed tomographic (SPECT) images were reconstructed using a filtered back projection algorithm with a Butterworth filter (cutoff: 0.15, order: 25) to reduce background noise artifacts. Reconstructed transaxial slices were 9.86 mm (2 pixels) thick and were aligned along the long axis of the dog in a caudad-cephalad orientation. Eight to 10 transaxial slices of each heart usually contained all the visualized indium-111 antimyosin activity. Indium-111 activity also was present in the liver, spleen and kidneys. To compare the relative activity of indium-111 antimyosin in the heterotopic and native hearts, the borders of the two hearts were first localized on the thallium-201 scans and regions of interest were drawn manually on the corresponding indium-111 scans for each heart from the transaxial slice that visually contained the highest counts. Background regions were also drawn. A ratio of the average counts per pixel (corrected for background) in the heterotopic compared with the native heart of each dog (heterotopic to native heart count ratio) was calculated.

Individual segments of each heart were dissected postmortem, as described under Pathologic Examination, and these segments were scanned by placing them on the face of one of the scintillation heads of the SPECT imaging system. Two static images were acquired for 15 minutes each using first the 240 keV photopeak of indium-111 and then the 70 keV photopeak of thallium-201. Regions of interest were drawn manually around the images of the tissue segments of both the native and the heterotopic hearts and heterotopic to native heart count ratios were calculated for comparison with the ratios obtained in vivo.

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Pathologic examination. Both hearts were removed and rinsed in normal saline solution before dissection. The atria and great vessels were discarded and each heart was divided into three sections, the right ventricular free wall, the interventricular septum and the left ventricular free wall. Representative small specimens were obtained for histopathologic examination from the right ventricle, left ventricle and septal sections and the remaining specimens were scanned on the camera face.

The tissue samples were fixed in 10% neutral buffered formalin and routinely processed for light microscopy. Five micron sections were cut and stained with hematoxylin-phloxine-saffron. Adjacent portions of fresh tissue for immunohistologic study were placed in OCT compound (Lab-Tek) in plastic BEEM capsules and snapfrozen in dry ice and isopentane, then stored at −70°C.

Rejection was graded semiquantitatively using an adaptation of the method of Billingham (12). The following scale was used: 0 = no rejection; 1 = mild mononuclear cell infiltrate; 2 = moderate mononuclear infiltrate with rare myocyte necrosis; and 3 = severe mononuclear infiltrate plus sheets of myocyte necrosis. The degree of rejection was graded from 0.5 to 3.0 without knowledge of the dog's treatment protocol or the results of the radionuclide scans. It should be noted that in patients receiving cyclosporine, the presence of a mononuclear infiltrate alone (grade 1) is not considered rejection requiring treatment. Only the presence of myocyte necrosis or a persistent heavy lymphocytic infiltrate, or both, is judged as treatable rejection (1,3). Tissue samples were taken from the anterior and posterior portions of right and left ventricular free walls and from the interventricular septum; thus, a minimum of six sections were examined from each heart. In addition, each free wall sample was divided into three zones (epicardium, midportion and endocardium) and each zone was assessed separately. The interventricular septum was also divided into zones (right and left endocardium and midportion). A final histologic rejection score was obtained by averaging the individual scores for all the zones in the right ventricle, septum and left ventricle for each heart.

Immunohistologic studies. These studies were also performed to determine if the indium-111 antimyosin was localized only to areas of myocyte necrosis. Tissues for immunoperoxidase study were maintained at −70°C until the time of staining. They then were removed from the BEEM capsules without thawing and were mounted on cryostat chucks with more OCT compound. Frozen sections, 5 μm in thickness, were cut, placed on glass slides and fixed for 2 minutes in acetone. Subsequent staining included incubation in 0.3% hydrogen peroxide in methanol to block endogenous peroxidase activity, then incubation in 10% normal goat serum in phosphate-buffered saline solution, pH 7.2, followed by incubation with a 25 μg/ml solution of peroxidase-conjugated goat anti-mouse IgG ($F[ab']_2$ specific). Antibody localization was finally demonstrated by incubation with a solution of 3,3-diaminobenzidine tetrahydrochloride (Sigma) at a concentration of 0.9 mg/ml in 0.03% hydrogen peroxide in modified phosphate-buffered saline solution (13), pH 7.4. Sections were counterstained with Light Green (Fisher Scientific), dehydrated, permanently mounted and examined with light microscopy.

Results

Acute Allograft Rejection

The planar and SPECT scans performed on the donor and recipient dogs with a normal heart before transplantation showed no significant uptake of indium-111 monoclonal antimyosin Fab (Fig. 1). Also, no uptake was visible in planar or SPECT images of the recipient's native heart after transplantation. In contrast, intense radioactivity was present in the scintigraphic images of the transplanted hearts of both Dog 1 (10 days after transplantation) and Dog 2 (4 days after transplantation) when there was electrocardio-

Figure 1. Dog 1. **A,** Baseline left lateral planar images of the donor dog for Dog 1 (thallium on the **left,** indium on the **right**). **B,** The cardiac region outlined was identified using the thallium image and shows the position of the heart on the indium scan. No significant indium activity is present in the heart before transplantation. **C,** The left lateral planar indium image of Dog 1, which received the heart shown in panel **A, left.** There is now intense uptake of indium in the region of the heterotopic heart superiorly that corresponded to histopathologic findings of acute severe rejection (compare with **A, right**). There is no activity in the native heart inferiorly.

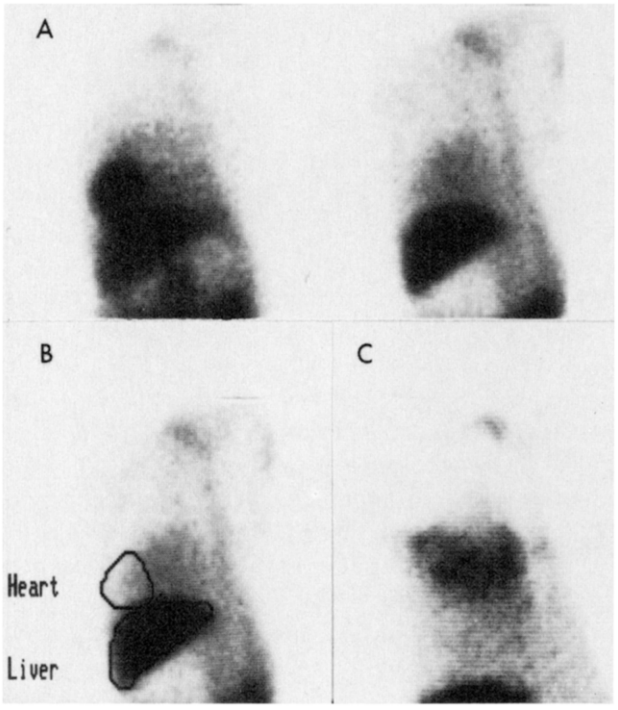


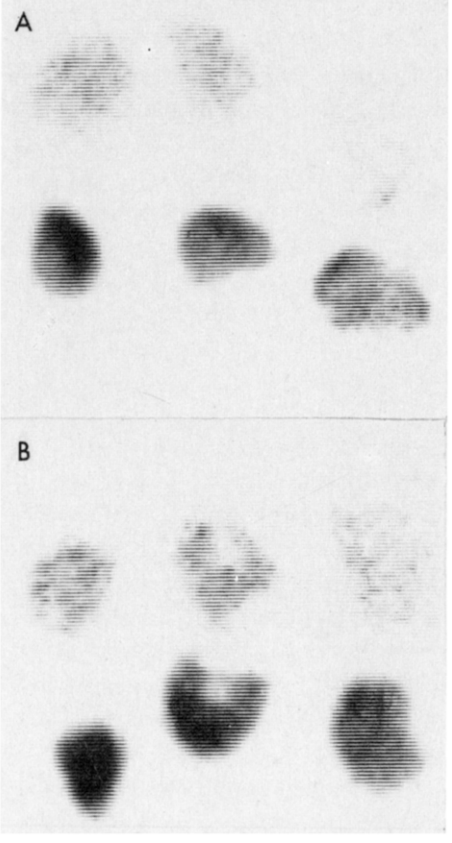
Table 2. Heterotopic to Native Heart Count Ratios* and Histopathologic Rejection Scores

	Baseline Scan Ratio	Rejection Scan Ratio	Excised Heart Ratio	Pathologic Rejection Score	Excised Heart Tissue Section Ratios
Acute					
Dog 1	—	4.39	7.69	2.5	RV > LV
Dog 2	—	4.44	7.35	2.5	LV > RV
Chronic					
Dog 3	1.79	—	1.68	0.25	LV > RV
Dog 4	1.08	2.32	2.85	1.06	LV > RV
Dog 5	1.40	5.70	6.90	2.75	RV > LV
Dog 6	2.39	1.89	1.82	0.58	LV > RV

*All ratios compare average counts/pixel of the heterotopic with the native heart in each dog. LV = left ventricle; RV = right ventricle.

graphic evidence of rejection (Fig. 1). Increased radioactivity was diffusely present throughout both ventricles of the heterotopic hearts. The heterotopic to native heart count ratios from these SPECT images were 4.39 for Dog 1 and 4.44 for Dog 2 (Table 2). The heterotopic to native heart count ratios for the excised hearts of Dogs 1 and 2 scanned on the camera face were 7.69 and 7.35, respectively. The count ratios for the individual tissue sections of each heart were highest for the right ventricle in Dog 1 and for the left ventricle in Dog 2. Figure 2 shows images of the cardiac sections on the camera face and demonstrates that all the indium activity is localized to the heterotopic heart, whereas the native heart shows only background activity. In addition, there are focal areas of more intense activity within some regions of the left or right ventricles.

Figure 2. Planar indium images of the six individual heart sections from Dog 1 (**A**) and Dog 2 (**B**). The heart sections are arranged from **left to right:** left ventricle, septum, right ventricle. Native heart sections are on the **top** of each group; heterotopic sections on the **bottom.** All three heterotopic sections show high counts compared with the native heart sections. There are focal regions of increased uptake within individual sections in both dogs. In Dog 2 (**B**), the indium activity ratio is greater in the left than in the right ventricle, whereas in Dog 1 (**A**) there is one focal area of intensity in the right ventricle that makes the overall count ratio greater for the right than for the left ventricle.



Histopathologic examination. Examination of the two native hearts showed no pathologic changes. The allograft in Dog 1 showed severe rejection (total score 2.5) in all portions of the right ventricle, left ventricle and septum; this was accompanied in the right ventricle by marked vasculitis. The rejection was histologically more severe in the right than in the left ventricle. In Dog 2, the allograft also showed moderate to severe rejection with an average score of 2.5. Examples of the histopathologic features of moderate and severe rejection are illustrated in Figure 3B and C. In this dog, the score was higher for the left ventricle than for the right ventricle or septum. There was no vasculitis evident in this allograft that had been transplanted for only 4 days.

Immunoperoxidase studies. The studies using peroxidase-conjugated goat anti-mouse IgG in these dogs (and also in the chronic dog model) demonstrated that indium-111 antimyosin was localized to areas of cardiac myocyte necrosis. Peroxidase staining showed a deposition of antimyosin antibody along the disrupted sarcolemmal surfaces of necrotic myocytes. No antibody was demonstrated in areas of interstitial edema in the absence of myocyte necrosis, in vessels disrupted by vasculitis or in normal myocardium.

Chronic Allograft Rejection

There was no clearly visible uptake of indium-111 antimyosin in planar or SPECT images of the native heart in the four dogs with a long-term cardiac transplant. Faint, diffuse uptake was apparent in the cardiac allograft of all

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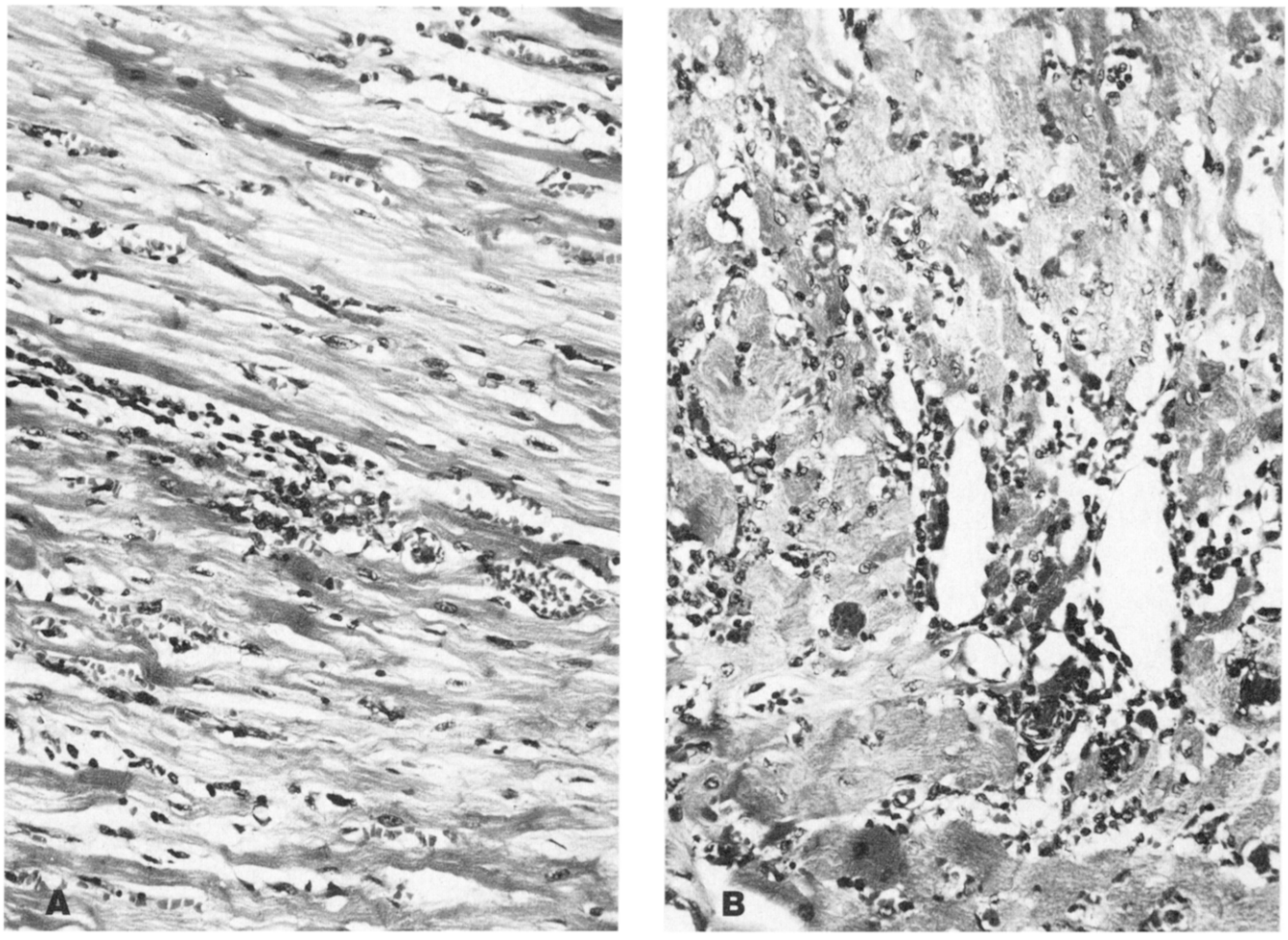


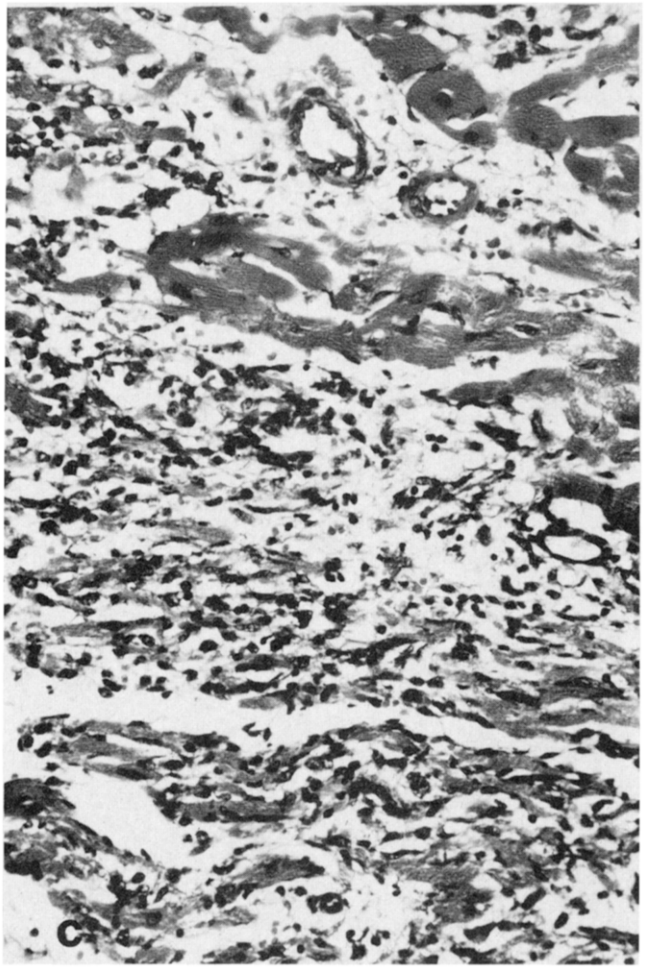
Figure 3. Light microscopic sections of heterotopic cardiac allografts. **A,** Mild rejection with interstitial lymphocytic cellular infiltrate; **B,** moderate rejection with more extensive infiltrate and focal myocyte necrosis; **C,** severe rejection with extensive necrosis of myocytes and cellular infiltrate.

of these dogs that received long-term immunosuppression, but intense indium-111 activity was found only in those with electrocardiographic evidence of rejection.

Dog 3. This dog was imaged and killed as a control while receiving immunosuppressive therapy. The heterotopic to native heart count ratio obtained from the SPECT images of the two hearts was 1.79 and the count ratio from the images of the excised specimens was 1.68. Histologic examination of the allograft showed negligible evidence of rejection with a total score of 0.25. An ancillary finding in the epicardial and larger intramyocardial vessels of the allograft was the presence of extensive, accelerated atherosclerosis. There were also areas of old infarct in the right ventricle, left ventricle and septum with myocyte dropout and healing with fibrosis. The immunoperoxidase studies did not show deposition of indium-111 antimyosin in these areas of old healed infarct.

Dogs 4, 5 and 6. These dogs were each studied twice to investigate the changes in scans that occur with rejection. The heterotopic to native heart count ratios from the SPECT images of Dog 4 were 1.08 at baseline and 2.32 16 days after immunosuppression was withdrawn. The heterotopic to native heart count ratio of the excised heart was 2.85. Light microscopy revealed mild rejection (Fig. 3A) with a total score of 1.06. In this heart, the left ventricle showed more intense rejection than did the right ventricle.

Figure 4 shows the scans of Dog 5 which received a transplant 9 months before study. The baseline study shows faint, diffuse indium-111 antimyosin uptake in the heterotopic but not in the native heart. When a repeat scan was performed 27 days after immunosuppression was withdrawn, the heterotopic heart displayed intense indium-111 uptake with a few focal areas of greater activity. The heterotopic to native heart count ratio from the SPECT images of this dog's heart was 1.4 at baseline and increased to 5.7 on the second scan obtained at the time of clinical rejection. The count ratio of the excised hearts was 6.9. In these hearts, the count ratios obtained from the images of the tissue sections were highest in the right ventricle. The pathologic examination showed extensive severe rejection in all sec-



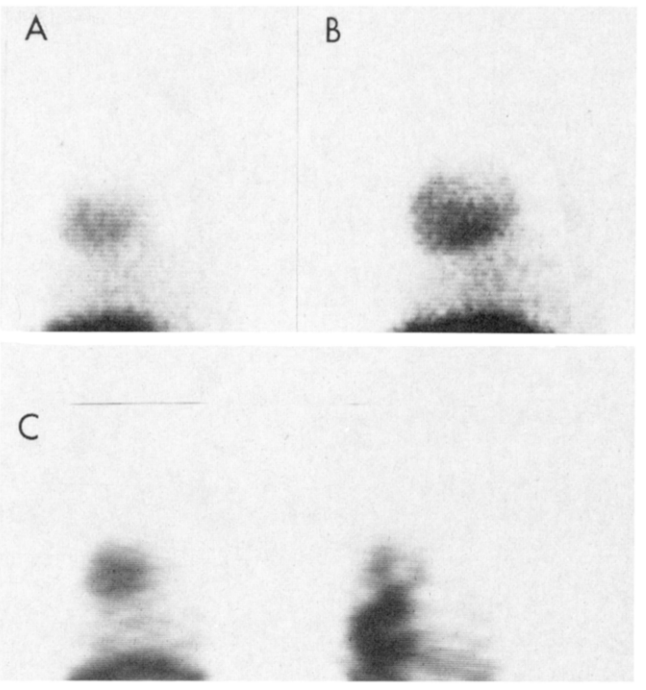


Figure 4. Dog 5. **A,** Baseline left lateral planar indium image shows faint diffuse uptake of indium in the region of the heterotopic heart. **B,** Left lateral planar indium image during allograft rejection 27 days after cessation of immunosuppression, showing more intense uptake in the transplanted heart. **C,** SPECT images from the baseline study. **Left,** A 1 cm thick midline long axis slice showing diffuse mild indium-111 uptake in the heterotopic heart. Note the contrast enhancement produced by the SPECT reconstruction compared with the planar image (**A**). The same slice from the thallium-201 scan on the **right** delineates the cardiac borders of both the heterotopic and native hearts.

tions with a total score of 2.75. A mild vasculitis was present in muscular vessels in the sections from the right ventricle, whereas a more marked vasculitis was present in the left ventricle and septum. In addition, there was acute ischemic change manifested as a coagulative necrosis of myocardial cells and necrosis of infiltrating cells without any polymorphonuclear cell reaction. The age of the infarct was therefore estimated at 12 to 48 hours. The infarct appeared to be due to the vasculitis of rejection, with subsequent thrombosis and infarction.

Dog 6 had evidence of spontaneous rejection by electrocardiogram at the time of the baseline scintigraphic study; this dog was continued on the same immunosuppressive medications for 2 weeks longer and was then restudied. Greater indium-111 uptake was present in the heterotopic heart on the baseline scan compared with the final scan. The heterotopic to native heart count ratio from the baseline SPECT image was 2.39, which decreased to 1.89 on the second scan 2 weeks later. In this dog, the heterotopic to native heart count ratio for the excised hearts was 1.82 and the ratio from the tissue sections was highest for the left ventricle. Histopathologically, there was very mild rejection predominantly in the left ventricle with a total score of 0.58. There was moderate allograft atherosclerosis and in the left ventricle there were numerous foci of healing in which myocardial tissue had been removed and replaced by mononuclear inflammatory cells, edema, granulation tissue and fibrosis. There was no evidence of active necrosis of myocardial fibers, which suggests that the focal healing may have represented resolving rejection or the repair that follows ischemic infarction. The histologic appearance suggested that the damage may have occurred 2 to 3 weeks before the dog's death, coinciding with the timing of the first study.

Comparisons. The heterotopic to native heart count ratios obtained from the in vivo SPECT images of both hearts in each dog before death correlated positively with the heterotopic to native heart ratios obtained when sections from the excised hearts were scanned on the face of the scintillation camera ($r = 0.93$) (Fig. 5A). When the total rejection scores obtained from the pathologic examination were compared with the SPECT ratios, an excellent correlation ($r = 0.97$) was demonstrated (Fig. 5B).

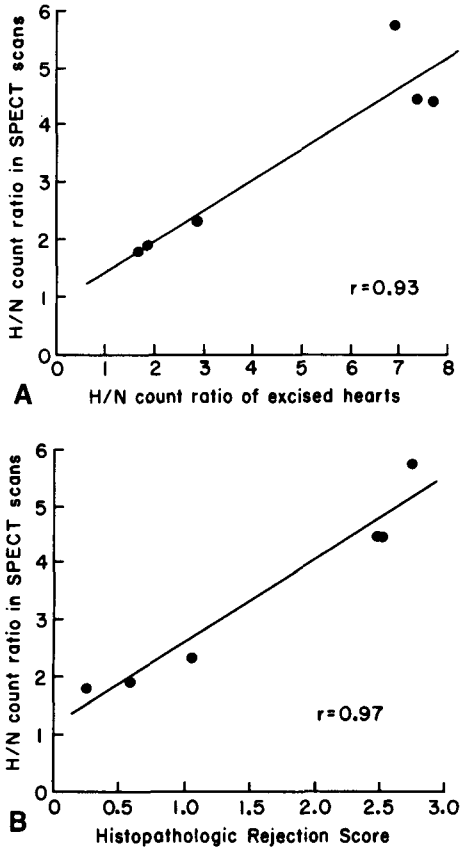


Figure 5. A, Comparison of heterotopic to native heart (H/N) count ratios in SPECT images (**ordinate**) and in vitro scans of tissue sections (**abscissa**). **B,** Comparison of H/N count ratios in SPECT images (**ordinate**) and histopathologic rejection scores (**abscissa**).

Discussion

Cardiac transplantation has become a more common therapeutic approach in recent years. Improved survival is judged to be secondary to the use of cyclosporine and prednisone for immunosuppression instead of the previous regimen of azathioprine and prednisone. However, the precise detection of allograft rejection remains a major problem in these patients. With azathioprine treatment, rejection was almost universally accompanied by myocardial edema and lymphocytic infiltrate, and therefore a drop in electrocardiographic voltage. In contradistinction, rejection in cyclosporine-treated patients is usually clinically silent, and current diagnostic techniques depend on evidence of myocyte necrosis on endomyocardial biopsy, which is performed routinely to detect rejection in its early phases (1,3).

Although utilization of this invasive surveillance technique in the clinical setting is undesirable because of inconvenience and some morbidity, other noninvasive measures (such as M-mode or two-dimensional echocardiography or high frequency averaging of the electrocardiogram) are indirect measures of the effects of cardiac rejection on heart function, whereas antimyosin uptake is a direct result of the myocyte necrosis on a cellular level (3–5). Previous radionuclide studies (14–16) have been performed using lymphocytes labeled with either indium or technetium in nonimmunosuppressed or azathioprine-treated rats. These studies showed increased cardiac radioactivity associated with cardiac rejection. However, because mild lymphocyte infiltration is a relatively nonspecific pathologic finding in cardiac transplant patients receiving cyclosporine, and more marked lymphocyte infiltration without myocyte necrosis unless persistent, is usually not regarded as an indication for additional therapy for rejection (1,3), these radionuclide techniques have not been widely used.

Detection of rejection. Our data indicate that SPECT imaging of indium-111 antimyosin is capable of detecting myocyte necrosis due to cardiac allograft rejection. There was no significant uptake of the radiolabeled antibody to cardiac myosin in the normal dogs scanned before surgery or in the native hearts of the dogs with acute or chronic rejection of the cardiac allografts. In contrast, there was intense uptake of indium-111 antimyosin in the cardiac allografts that exhibited electrocardiographic or histologic evidence of rejection. When the myocardial tissues were counted postmortem, there was excellent correlation between the ratios of counts in the heterotopic and normal hearts and those obtained from the tomographic reconstructions of the in vivo SPECT imaging. The SPECT count ratios also correlated highly with the histopathologic scoring of the multiple tissue samples used to grade the severity of the rejection process. These results indicate that the quantitative data obtained from the noninvasive SPECT images of indium-111 antimyosin uptake were indicative of the severity of myocyte necrosis due to acute or chronic rejection of the heterotopic hearts. This conclusion was reinforced by the histopathologic studies with immunofluorescence and peroxidase. These studies revealed the presence of the anticardiac myosin Fab only in areas of myocyte necrosis. There was no apparent localization of the Fab in normal myocardium, in areas of interstitial edema or in the blood vessels disrupted by vasculitis.

To investigate whether SPECT imaging with indium-111 antimyosin could detect serial changes over time, three dogs were each studied twice, before and after the development of rejection. Two of these dogs (Dogs 4 and 5) with no clinical evidence of rejection before their first scan developed electrocardiographic signs of rejection after their immunosuppression was discontinued. The ratios of the indium-111 antimyosin uptake in the heterotopic to native hearts of these dogs increased proportionately to the degree of histopathologic rejection found at death. The third dog (Dog 6) had evidence of spontaneous rejection by electrocardiogram at the time of the baseline study. Immunosuppression was continued at the same dosage and when SPECT scanning of this dog was repeated there was de-

creased indium-111 antimyosin uptake compared with that at the baseline study, accompanied by pathologic evidence of resolving allograft rejection. These data imply that a decreasing heterotopic to native heart ratio on scanning may be a sign of resolving damage. Serial imaging with indium-111 antimyosin may be useful in following the responses of cardiac allografts to treatment for rejection.

Pattern of rejection. In the present study, evidence of allograft rejection by in vitro scanning (indium-111 antimyosin uptake) and histopathologic examination was found to be greater in the left than in the right ventricle in four of the six dogs. Other workers have also found an asymmetric pattern of rejection. Haverich et al. (17) studied primates after orthotopic cardiac transplantation. Although they found histologic evidence of rejection to be more severe in the right than in the left ventricle, there was a lesser degree of rejection in the right ventricular endocardium compared with its midlayer, and less still in the right septal midlayer compared with the right ventricular free wall. The discrepancy between our results and those of these investigators may be due to differences in coronary circulation between primates and dogs, our small sample size or the use of a heterotopic model in our study. However, in both studies an asymmetric pattern of rejection was observed and in our study it was detected by antimyosin antibody. In current clinical management of patients with a cardiac transplant, it is assumed that the degree of rejection found on endomyocardial biopsy from the right ventricular septum reflects the degree of rejection throughout the entire heart. Biopsies, however, which sample only very small areas of the endocardial surface, may easily miss patches of rejection present elsewhere in the heart and may only detect rejection when it is sufficiently advanced to involve the entire right ventricle. In our in vivo studies with SPECT, it was difficult to distinguish the right and left ventricles because of the anatomic distortion created by placing the cardiac allograft in each host dog's chest. However, in orthotopic transplants it should be possible using SPECT to identify the cardiac chambers and to detect cardiac rejection scintigraphically when it occurs in areas other than the right ventricular septum.

Low grade cardiac rejection and accelerated coronary atherosclerosis. Low grade cardiac allograft rejection that is not detected by electrocardiogram or by routine endomyocardial biopsy may be present in animals or in patients maintained on long-term immunosuppressive therapy. Long-term follow-up of cardiac transplant patients on cyclosporine immunosuppression has shown evidence of increased intramyocardial fibrosis and accelerated coronary atherosclerosis in many. Although the pathogenesis of these changes is uncertain, they are thought to be a consequence of recurrent immunologic damage to the endothelium of the coronary vessels that occurs during undetected low level rejection (18). In our study, a faint uptake of indium-111 antimyosin was observed in the chronically transplanted hearts of Dogs 3, 4 and 5 in the first study while they were receiving immunosuppressive therapy and had no electrocardiographic evidence of rejection. Although histologic confirmation of rejection was not obtained until after the second study in Dogs 4 and 5, when drugs were withdrawn, the finding of low grade anticardiac myosin uptake in the immunosuppressed dogs suggests these animals may have had a very low level of rejection. The extensive coronary vascular disease present in some chronically immunosuppressed dogs in our study is compatible with this speculation. Graft atherosclerosis was apparent in two of our chronically transplanted dogs and by itself did not seem to increase indium-111 antimyosin uptake. Old healed infarction as seen in Dog 3 also did not increase indium-111 uptake. We can only speculate that antimyosin antibody imaging may also be able to detect small silent myocardial infarcts that can occur secondary to the premature atherosclerosis.

Limitations of imaging technique. There are several disadvantages of indium-111 antimyosin imaging in its current state of development: 1) Because the tracer clears slowly from the blood pool, optimal images of the myocardium cannot currently be obtained until 24 to 48 hours after intravenous injection. 2) The long half-life and high energy of indium-111 increases the radiation burden to the main target organ, the kidney, which limits the dosage per injection and the number of possible injections per year. Use of other radionuclides such as iodine-123 to label the antimyosin may reduce the radiation burden to the patient from serial imaging. 3) Although Fab fragments are less likely to cause allergic reactions than are whole antibodies (because of more rapid excretion by the kidneys), there is a small but significant possibility of allergic or hypersensitive reactions associated with multiple injections of foreign protein. Pertinent to this possibility, however, are data from a recent multicenter trial (19) in which a murine OKT_3 monoclonal antibody was administered repeatedly to 197 patients with a renal transplant for 14 days as therapy for acute rejection with no episodes of anaphylaxis, hypersensitivity or serum sickness. 4) Because antimyosin antibodies are taken up by necrotic myocytes, indium-111 antimyosin imaging would not distinguish between rejection and the coagulative necrosis that can be seen on the first posttransplant biopsy of patients receiving high dose vasopressors or those with distantly obtained hearts. For this reason, we envision this technique to be more useful in the later follow-up period.

Conclusions. Our data show that SPECT and planar imaging of indium-111 antimyosin can detect, localize and quantify cardiac allograft rejection in dogs with a heterotopic cardiac transplant. They suggest that in patients with an orthotopic cardiac transplant, noninvasive detection and quantitation of rejection from planar and SPECT indium antimyosin scans may be possible. Obviously, to obtain quantitative data concerning uptake of indium activity in a

heart in the orthotopic position, corrections for attenuation and background activity would be necessary. A prospective study comparing SPECT indium-111 antimyosin imaging with results of endomyocardial biopsy for the detection of cardiac transplantation rejection is currently under way in our institution. If successful, this approach will have the advantage of being noninvasive and could potentially decrease the number of endomyocardial biopsies performed on the patients after cardiac transplantation.

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